EXHIBIT

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Polymer Science and Materials Chemistry

Exponent®

Expert Report of Dr. Steven MacLean

United States District Court
For The Southern District Of
West Virginia
Charleston Division

This document relates to:
Ethicon Inc., Pelvic Repair System Products Liability Litigation

Exponent®

Expert Report of
Dr. Steven MacLean

**UNITED STATES DISTRICT COURT
FOR THE SOUTHERN DISTRICT OF
WEST VIRGINIA
CHARLESTON DIVISION**

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Polymer Science and Materials Chemistry

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Limitations

At the request of Butler Snow LLP, Exponent reviewed relevant scientific literature, historic documented studies and expert reports for the pending litigation. Exponent investigated specific issues relevant to this report as requested by the client. The scope of services performed during this investigation may not adequately address the needs of other users of this report, and any reuse of this report or its findings, conclusions, or recommendations is at the sole risk of the user. The opinions and comments formulated during this investigation are based on observations and information available at the time of the investigation.

The findings presented herein are made to a reasonable degree of engineering certainty. We have endeavored to be accurate and complete in our assignment. If new data becomes available or there are perceived omissions or misstatements in this report, we ask that they are brought to our attention as soon as possible so that we have the opportunity to address them.

Steven MacLean, Ph.D., P.E. Biography

I am a Senior Managing Engineer in the Polymer Science and Materials Chemistry Practice at Exponent Failure Analysis Associates, Inc. ("Exponent"). My expertise and experience includes the chemical and physical behavior of polymeric materials in end-use applications, specifically in the evaluation of polymeric components in product safety assessments and product failure analysis. I have a B.S. and M.E. in Mechanical Engineering from Rensselaer Polytechnic Institute, and a M.S. in Material Science and Engineering from Rochester Institute of Technology. I also obtained a Ph.D. in Material Science from the University of Rochester in 2007. I am a registered Professional Engineer in New York and Maryland, a Senior Member of the Society of Plastics Engineers (SPE), and a board member of SPE's Failure Analysis and Prevention Special Interest Group.

During the pursuit of my advanced degrees in materials science, my chosen field of study was polymer science and engineering. Graduate courses taken during my academic career that specifically focused on polymers included, but were not limited to, polymer science, organic polymer chemistry, polymer physics, polymer rheology, polymer processing, bulk physical properties of polymers, adhesion theory, and analytical techniques for polymeric materials. Supplemental course work included mechanics of materials, fracture mechanics, thermodynamics of materials and electron microscopy practicum. At the master's degree level, my polymer research included characterizing the changes in chemical and physical properties of polycarbonate due to multiple heat histories from processing. At the doctoral level, my polymer research was focused on developing and investigating novel formulations of rubber-toughened polyphenylene ether polymers for use in pressurized, potable water systems. The primary emphasis of my dissertation included quantifying changes in select mechanical properties, including fracture toughness and tensile properties, due to the degrading effects from persistent exposure to chlorinated water at elevated temperatures.

In addition to my academic education and training, I have also been actively practicing in the field of polymer science and engineering for the past 20 years. Throughout that time, I have routinely utilized numerous polymer characterization techniques including, but not limited to, infrared spectroscopy, chromatography, mass spectrometry, calorimetry as well as optical,

scanning electron and transmission electron microscopy. In particular, I have used these microscopic techniques to examine the topography and morphology of fracture surfaces created as a result of polymer cracking. I have also employed these techniques to characterize modes of polymer failure such as creep, fatigue, stress overload, and environmentally-assisted stress cracking. In many instances, I have published the use of these analytical techniques to investigate polymer failures in commercialized products in peer-reviewed journal articles and scientific conference proceedings.

Prior to joining Exponent in 2011, I worked for over 15 years at General Electric Plastics (GE) and SABIC Innovative Plastics (SABIC) in a variety of technical roles of increasing responsibilities. Throughout my tenure, I was routinely involved in material selection, performance and testing for, among other things, high-demand applications, product safety assessments, and product failure analysis. As a result I have significant expertise and experience with industry standards and applicable regulations that prescribe the technical performance of polymeric materials in end-use applications, including those in the medical device industry.

At GE Plastics, I was trained extensively in the Six Sigma quality process, and became certified as a Six Sigma Black Belt. As a Certified Six Sigma Black Belt, my responsibilities included improving business processes by employing a variety of well-established statistical methods as well as mentoring and training Six Sigma Green Belts throughout the company.

Throughout my career, I have evaluated the suitability and performance of polymeric materials in end-use applications, including specifically, for the medical device industry. While at GE and SABIC, I worked with numerous medical device companies on material development, material specification, design and manufacturing for a wide variety of medical device applications. These efforts included, inter alia, developing and implementing tests related to the bulk physical properties of polymeric materials specified in said devices as well as material formulation development to meet unique device requirements that could not be met with off-the-shelf grades of resin. Formulation development often included the selection and refinement of base polymers or alloys, molecular weight, additives, stabilizers, processing aides, lubricants, colorants and inorganic fibers and fillers. In addition to proactive design and material selection assistance, I have worked on hundreds of product safety assessments and failure analyses

involving polymeric materials, many of which were performed on medical devices and components.

In my prior role as Director of Global Agency Relations and Product Safety at GE/SABIC, part of my leadership responsibilities included being an active member of the business' Healthcare Resins Advisory Board. The board developed internal processes and standards for the specification, use and sale of GE/SABIC resins in medical device applications. These efforts included ensuring that commercial resin grades within the GE/SABIC healthcare portfolio were assessed for biocompatibility using industry accepted test protocols such as United States Pharmacopeia (USP) Class VI, Tripartite Biocompatibility Guidance or ISO 10993 Biological Evaluation of Medical Devices standards. For the past several decades, the latter two standards have been supported by the Food and Drug Administration (FDA) and commonly employed to assess the potential for cytotoxicity, hemolysis, pyrogenicity, sensitization issues, among other biological effects, when the human body is exposed to foreign materials. In addition, the board also ensured that "good manufacturing processes" were globally implemented to maximize the purity levels of all compounded materials within the healthcare resin portfolio.

In addition to my relevant training, education and industry experience, I have also reviewed and synthesized the available public literature pertaining to *in vivo* and *in vitro* studies of polymeric mesh devices, long-term implantation of polymeric medical devices, foreign body response to implantable materials, as well as select plaintiff reports which allege *in vivo* PROLENE mesh degradation.

Background

Introduction

Plaintiff's expert, Dr. Vladimir Iakovlev, opines that Ethicon's PROLENE mesh degrades after implantation as manifested by formation of cracks, nanopores, and nanocavities and a resultant increase in mesh stiffness.[1] Dr. Iakovlev bases his conclusions on flawed experiments in which he purports to show that oxidized, degraded PROLENE is stained using histological dyes. Dr. Iakovlev's conclusions are further flawed because he conducts no experiments to measure or otherwise quantify the alleged increase in mesh stiffness caused by the PROLENE degradation. The purpose of this report is to address the scientific deficiencies of Dr. Iakovlev's experiments and conclusions from a polymer science perspective; this report does not address issues related to histology[2] or Dr. Iakovlev's histological analysis.

PROLENE Mesh

Ethicon's antioxidant-stabilized polypropylene-based resin is known by the tradename PROLENE. The resin was determined to be "safe and effective for use" in nonabsorbable surgical sutures by the FDA in 1969,[3] and has been used ever since. PROLENE sutures are manufactured by a melt spinning process.[4] In addition to sutures, Ethicon has knit PROLENE filaments into mesh materials used in hernia repair and to treat pelvic organ prolapse.

[1] Expert report of Dr. Vladimir Iakovlev dated May 23, 2015, p. 54.

[2] Histology relates to the microscopic study of tissue. Here within, I focus on the microscopic study of PROLENE polymer fibers, although the chemistry of histological staining is discussed from a background perspective.

[3] NDA – 4.16.1969 PROLENE FDA Approval (ETH.MESH.09625731-09625737)

[4] FDA – Reclassification.pdf (ETH.MESH.10665538 – 1065565)

PROLENE Composition

As with many commercially available resin compounds, Ethicon's PROLENE resin is comprised of several raw material ingredients in addition to the base isotactic polypropylene. The additional formulation ingredients and corresponding loading level ranges are:[5]

- Calcium Stearate – 0.25-0.35% - lubricant to help reduce tissue drag and promote tissue passage
- Santonox R – 0.10-0.30% - a primary hindered phenol antioxidant
- Dilauralthiodipropionate (DLTDP) – 0.40-0.60% - a secondary thioester antioxidant
- Procol LA-10 – 0.25-0.35% - lubricant to help reduce tissue drag and promote tissue passage
- Copper Phthalocyanate (CPC) Pigment – 0.55% max - colorant to enhance visibility (in blue filaments only)

A summary of the full resin history including information on compounding, manufacturing, and formulation changes can be found in Karl's memo entitled "PROLENE Resin Manufacturing Specifications."[6]

Hematoxylin and Eosin (H&E) Stain

Hematoxylin and Eosin, also referred to as H&E, is a common stain used for illuminating components of cells and tissue, many of which are long molecules (polymers). The hematoxylin dye solution itself is a mixture of hematoxylin, hematein, aluminum ions, and solvent. It is used in combination with a "mordant" compound that helps link it to the tissue. This mordant is typically a metal cation, such as aluminum. This complex is cationic (positively charged) and can react with negatively charged, basophilic cell components, such as nucleic acids in the nucleus, rough endoplasmic reticulum, ribosomes, and acidic mucin. Eosin, used in combination with Hematoxylin, is negatively charged and attracts positively charged molecules.

[5] John Karl's January 23, 2003 Memo titled PROLENE Resin Manufacturing Specifications (Eth.Mesh.02268619 – 02268621)

[6] Ibid.

It stains structures with positive charges, e.g. cellular membranes, cytoplasm, connective tissue, and extracellular matrix tissue.

The mechanism for H&E "staining" of biological compounds is simple ionic bonding between two charges: charge on the H&E staining molecules and charges on the biological molecules. As an example, amino acids are the molecular building blocks of proteins (which are also polymeric) and some of these amino acids contain a charge as shown in Figure 1.[7] These charged compounds will bind ionically[8] (charge-to-charge) with H&E and appear stained. Ionic bonding is the most important type of bonding that occurs in histological staining techniques,[9] and similarly, the published mechanism for how H&E stains is not physical in nature. In other words, physical voids, cracks, or crevices in PROLENE (as posited by Dr. Iakovlev) or other materials do not "trap" or hold H&E stain, especially after washing and rinsing. In addition, it has been suggested that simple electrostatic forces alone are not sufficient to account for the staining of nuclei with Al/hematein solutions.

From a polymer science perspective, polypropylene or PROLENE, molecules that are not charged, are not expected to stain with H&E. Furthermore, as shown in my expert report, which discusses the pathways of polypropylene oxidation, oxidized polypropylene does not possess a distinct charged region. Therefore, in accordance with not only first principles of polymer science, but also the accepted methodology and assessment routinely reported in the literature, oxidized polypropylene is not expected to stain with H&E.

[7] Myers, R. (2011). The Basic Chemistry of Hematoxylin. Available from: http://www.leicabiosystems.com/pathologyleaders/the-basic-chemistry-of-hematoxylin/

[8] A common, familiar ionically bonded material is sodium chloride, or table salt, in which Na^+ and Cl^- are bound together by ionic attraction.

[9] Veuthey, T., Herrera, G., & Dodero, V. I. (2014). Dyes and stains: from molecular structure to histological application. Frontiers in bioscience (Landmark edition), 19, 91.

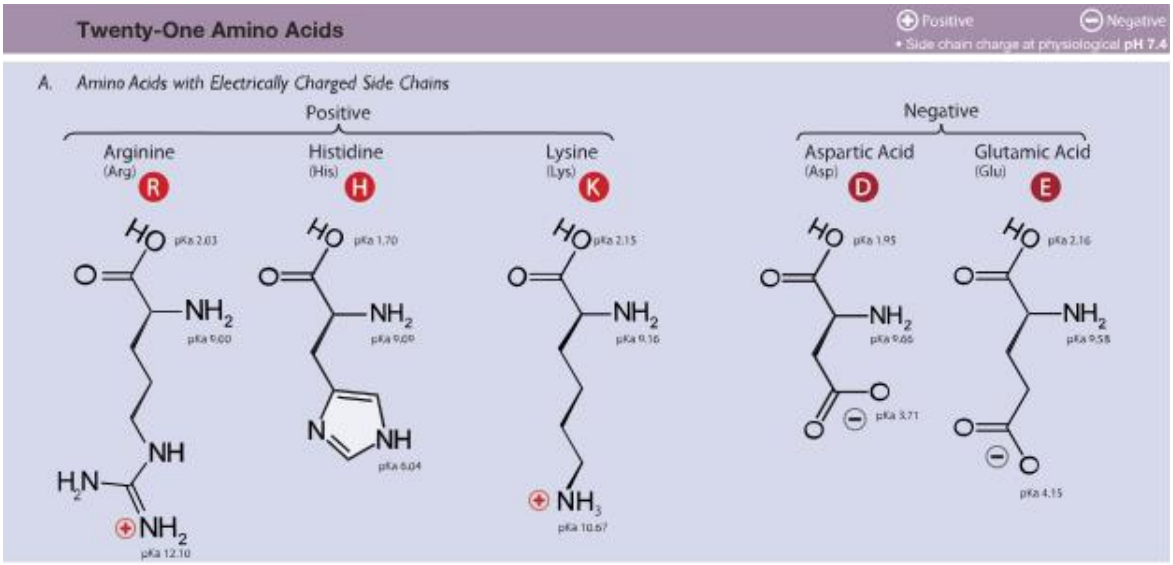

Figure 1. Amino acids that contain a net positive or negative charge.

Experimental Investigation of the Capacity of PROLENE and Oxidized PROLENE to Accept H&E Stain

In addition to my reliance on the literature and first principles of polymer science, in order to further validate my assertion that H&E is not expected to stain PROLENE or oxidized PROLENE, Exponent conducted a set of laboratory experiments as described below.

Sample Preparation Prior to Sectioning

Exemplar PROLENE Mesh

Pristine PROLENE mesh (Ethicon TVT Device, Ref. No. 810041B, Lot No. 3661669) was received and kept in its original packaging until use. A clean razor blade was used to cut sections for laboratory analysis.

Chemically Oxidized PROLENE Mesh

Sections of PROLENE mesh were oxidized according to the protocol published by Guelcher and Dunn.[10] Samples were incubated at 37°C for up to 5 weeks in oxidative media composed of 0.1 M $CoCl_2$ in 20 wt% H_2O_2. This solution purportedly simulates the oxidative environment created by macrophages in response to a foreign object.[11] The oxidative solution was changed every 2-3 days. Prior to processing, the samples were copiously rinsed in de-ionized water, air-dried, and assessed for morphological changes using scanning electron microscopy (SEM).

QUV Oxidized PROLENE Mesh

Sections of PROLENE mesh were placed inside a Q-Lab QUV Accelerated Weathering Tester and irradiated with 0.98 ($\frac{W}{m^2}$) UV-A and UV-B at 60°C for 5 days. As with the chemically oxidized meshes, the samples were assessed for morphological changes using SEM prior to processing.

[10] Guelcher, S. A., & Dunn, R. F. (2015, June). Oxidative degradation of polypropylene pelvic mesh in vitro. International. Urogynecology Journal. 26 (Suppl 1): S55-S56.

[11] Ibid.

Sample Preparation

Exemplar and oxidized mesh samples were embedded in both paraffin and resin (Technovit), sectioned, and stained with Hematoxylin & Eosin. All processing was performed by an independent histology lab. Detailed embedding and staining protocols can be found in Appendix A.

Paraffin-embedded samples were prepared by following the protocol submitted by Dr. Iakovlev. Briefly, samples were sequentially dehydrated in reagent alcohol and Xylene substitute using an automated tissue processor, then embedded in Leica EM400 Paraffin wax. Sections of the paraffin blocks (4-6 μm thick) were obtained using a microtome, briefly floated in a 40-45^{o}C water bath, then mounted onto slides. Sections were air-dried for 30 minutes then baked in a 45-50^{o}C oven overnight.

Resin-embedded samples were sequentially dehydrated in reagent alcohol using an automated tissue processor, then embedded in Technovit 7200. The polymerized resin block was trimmed, cut, and ground to a thickness of approximately 50 μm.

Paraffin and resin-embedded samples were stained with Aqueous Eosin and Harris Hematoxylin using an automated stainer. All slides were imaged by Exponent personnel using a microscope equipped with polarizing filters.

Results

SEM on Oxidized Meshes

When viewed under a Scanning Electron Microscope, the QUV-oxidized mesh exhibited external cracking (Figure 2), while the chemically-oxidized mesh did not (Figure 3). The observations on the latter mesh differ from the results published by Guelcher and Dunn, who

reported "pitting" and "flaking" in polypropylene meshes subjected to the same treatment conditions.[12]

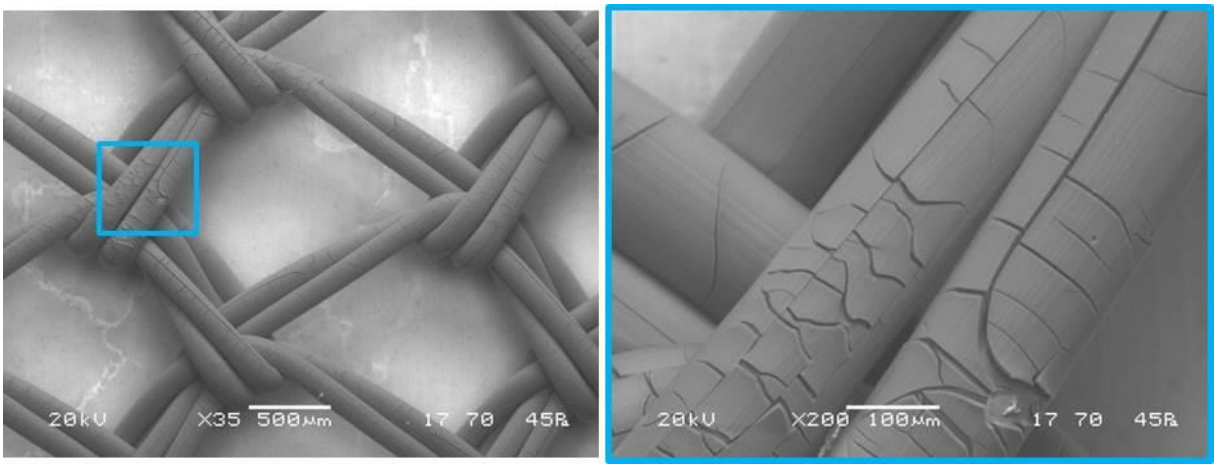

Figure 2. Scanning Electron Microscope images of QUV oxidized mesh.

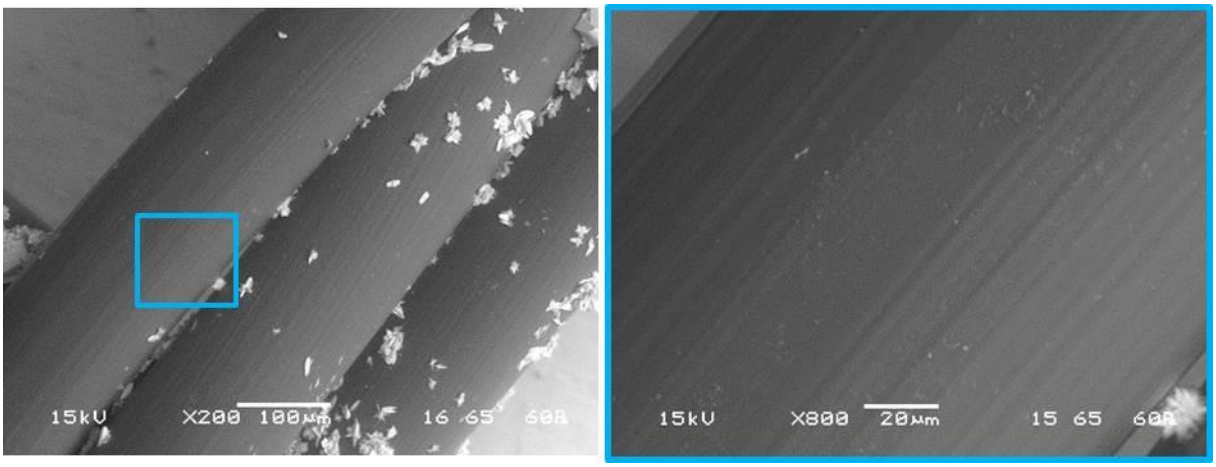

Figure 3. Scanning Electron Microscope images of mesh that was chemically-oxidized according to the Guelcher protocol.

[12] Guelcher, S. A., & Dunn, R. F. (2015, June). Oxidative degradation of polypropylene pelvic mesh in vitro. International. Urogynecology Journal. 26 (Suppl 1): S55-S56.

Intentionally Oxidized PROLENE Meshes Were Not Stained by the Hematoxylin & Eosin Dyes

Positive Control – Rabbit Skin

A positive control (rabbit skin tissue) was included with the mesh samples and processed simultaneously in the automated tissue stainer, to demonstrate the effectiveness of the protocol. PROLENE meshes were subjected to the staining protocol in the same batch.

The appearance of stain is evident when tissue is present and stain has been applied. Figure 4 shows the stark contrast between rabbit tissue that has not been treated with stain (left) and rabbit tissue that has been treated with stain (right). These results confirm that the staining protocol employed in these experiments is effective in staining proteinaceous materials.

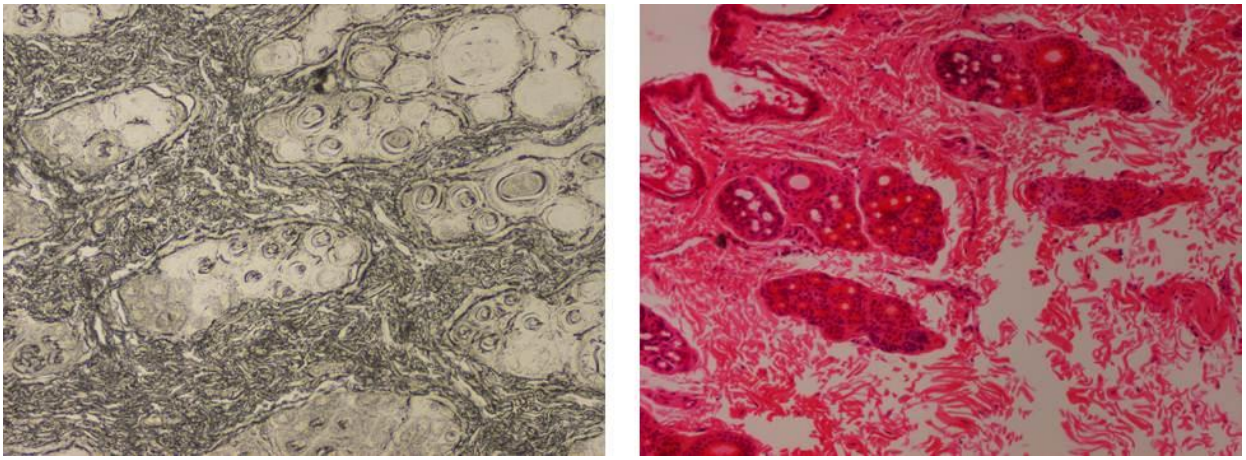

Figure 4. Processed and sectioned tissue not stained (left) and tissue that has been stained (right) are shown.

Non-Oxidized Control – Out-of-the-Box PROLENE Mesh

Exemplar PROLENE mesh samples with no prior exposure to laboratory UV or chemical oxidation were subjected to the Iakovlev staining protocol. As expected, the H&E stain did not bond to the PROLENE as displayed in Figure 5, confirming that the staining protocol is not effective in staining non-proteinaceous or non-ionic materials.

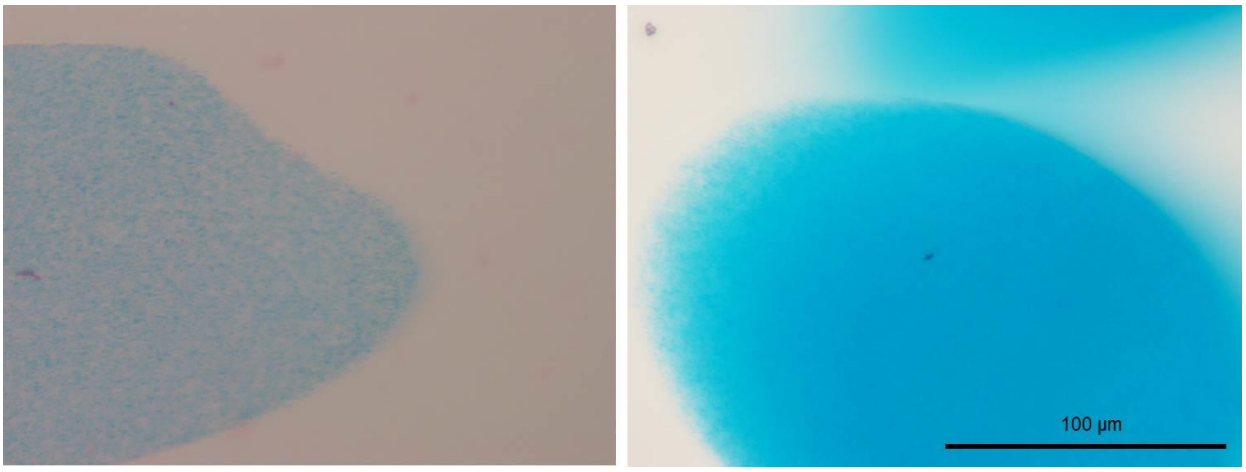

Figure 5. Pristine (exemplar) mesh embedded in paraffin (left) and resin (right), stained with Hematoxylin & Eosin.

Intentionally Oxidized PROLENE – Chemical Oxidation

Exemplar PROLENE mesh samples exposed to the Guelcher chemical oxidation procedure were also subjected to the Iakovlev staining protocol. As shown in Figures 6, 7, and 8, the chemically oxidized PROLENE did not accept the H&E stain, thereby confirming the flawed methodology of Dr. Iakovlev.

An additional observation made during these experiments was that manipulation of the polarizers could create a “bark”-like appearance on the fiber exterior (Figures 6 and 7). This effect is likely caused by the variant thickness of the fiber across its diameter as an artifact of the sectioning process. Interestingly, what Dr. Iakovlev describes as PROLENE dye particles can be seen in the false “bark”.

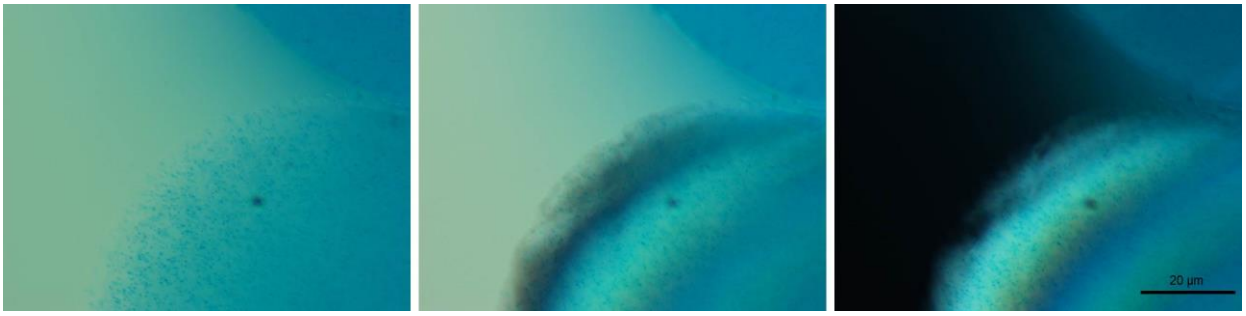

Figure 6. PROLENE mesh chemically oxidized mesh with the Guelcher protocol, embedded in resin, and subjected to the H&E staining protocol. Non-polarized light (left), plane-polarized light (center), cross-polarized light (right). No staining is evident.

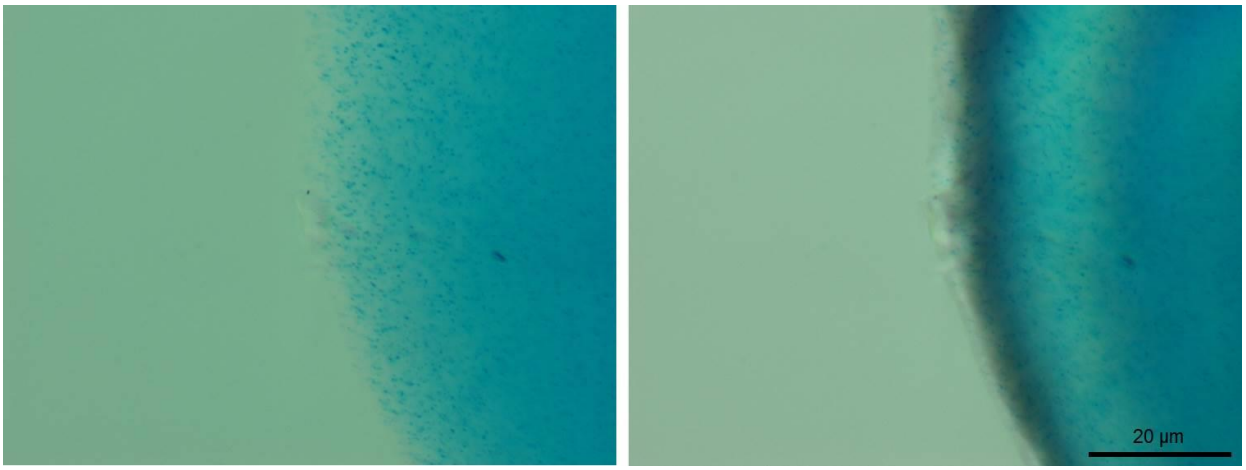

Figure 7. PROLENE mesh chemically oxidized mesh with the Guelcher protocol, embedded in resin, and subjected to the H&E staining protocol. Non-polarized light (left), plane-polarized light (right). No staining is evident.

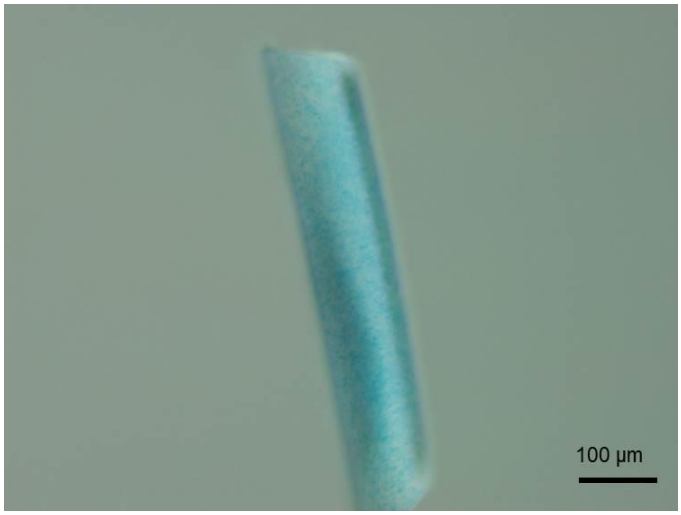

Figure 8. PROLENE mesh chemically oxidized mesh with the Guelcher protocol, embedded in paraffin, and subjected to the H&E staining protocol No staining is evident.

Intentionally Oxidized PROLENE – UV Oxidation

Exemplar PROLENE mesh samples exposed to QUV oxidation were also subjected to the Iakovlev staining protocol. As shown in Figure 9, despite the fact that it was cracked, and should have trapped stain according to Dr. Iakovlev, the QUV oxidized PROLENE did not accept the H&E stain, thereby confirming Dr. Iakovlev's flawed methodology. Despite multiple observations using high and low magnifications, polarized and non-polarized light, no evidence of the stain being trapped, captured, or otherwise bound within the cracks of the damaged mesh was observed.

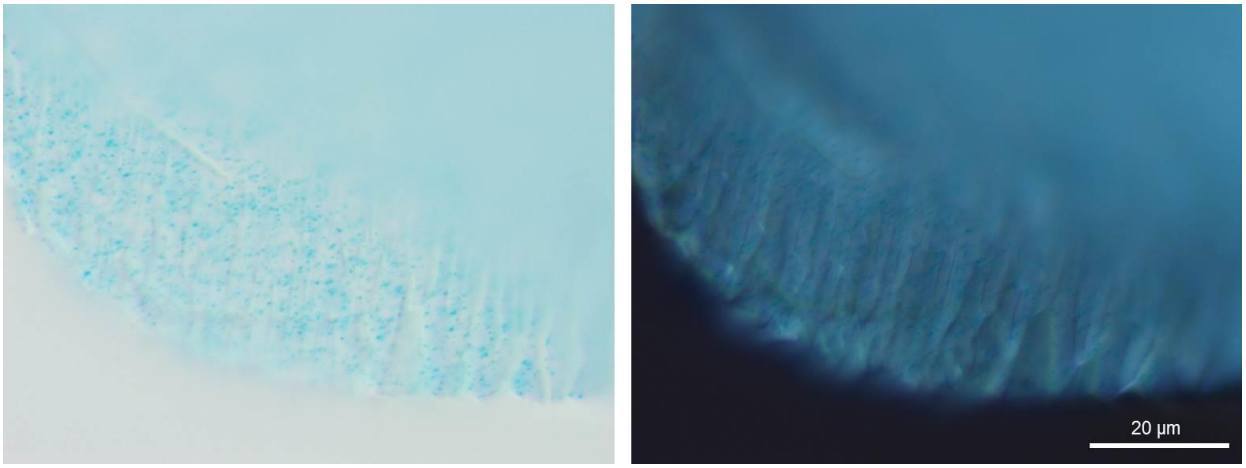

Figure 9. QUV treated mesh exhibiting several cracks but no evidence of H&E stain. Image on the left was acquired in absence of polarization, the image on the right was taken with polarization.

Imaging Artifacts

Microtome slicing of polymeric samples is a technique with which I am familiar, and has been used in the field of polymer science for decades.[13,14,15] Observation of thin-sliced polymeric specimens, including those that have been dyed, requires an understanding of potential artifacts that can exist as a result of the cutting process.

Thickness Variation and Stain Pooling

When high aspect ratio samples (such as fibers) are sectioned with a microtome, simple geometry dictates that the thickness will be variant if the microtome knife is not orthogonal to the sample's long axis. This geometric artifact is exhibited schematically in Figure 10A-10D, which illustrates that the edges of the sliced specimen are thinner when viewed under the microscope.

[13] Wang, X., & Zhou, W. (2002). Glass transition of microtome-sliced thin films. Macromolecules, 35(18), 6747-6750.

[14] Stiftinger, M., Buchberger, W., & Klampfl, C. W. (2013). Miniaturised method for the quantitation of stabilisers in microtome cuts of polymer materials by HPLC with UV, MS or MS2 detection. Analytical and bioanalytical chemistry, 405(10), 3177-3184.

[15] Janeschitz-Kriegl, H., Krobath, G., Roth, W., & Schausberger, A. (1983). On the kinetics of polymer crystallization under shear. European polymer journal, 19(10), 893-898.

This same effect can result in stain pooling, which is also illustrated schematically in Figure 10. The cylindrical fibers that compose the mesh (A) can be cut in an oval shape depending on the angle at which the blade encounters the block (B). When the resulting section (C) is placed upon a glass slide and stained, the angle between the section and the glass forms a small pocket in which stains can accumulate (D), giving the appearance of "true" staining (E) – that is, of chemical interactions between dyes and their ligands. In reality, this is merely a mechanical entrapment of the staining solution.

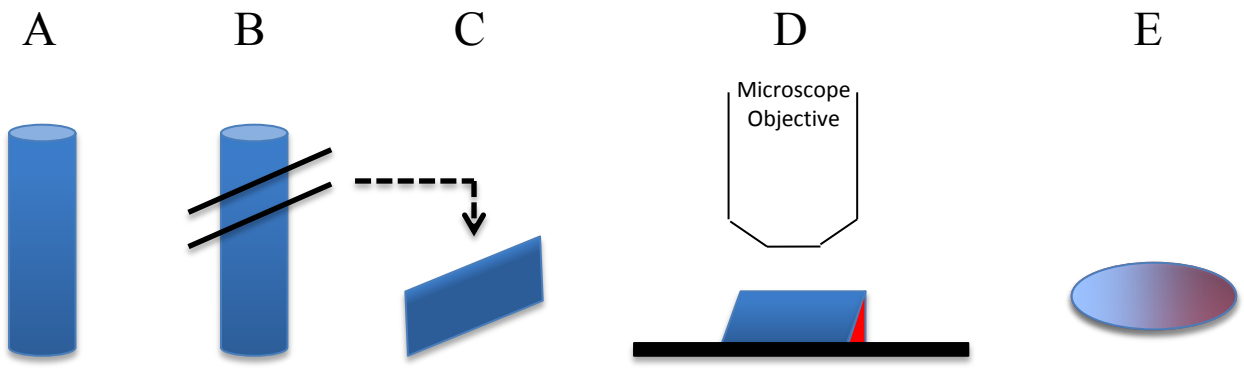

Figure 10. Potential formation mechanism of pooling artifact. A mesh fiber (A) can encounter the microtome blade at an angle (B), forming a section with an angled ledge (C), under which stain can pool (D) and give the appearance of true staining (E).

Polarizing Artifact

Polarized microscopy is a powerful tool in polymer science.[16] With good optics and proper alignment, it allows for the visualization of anisotropic structures, making them appear under varying shades of brightness with a polarizing filter in the microscope's light path.[17] The brightness of the sample when imaged under polarization depends on factors such as sample alignment. The brightness is highest when the object is aligned at a 45^{o} angle to the polarizers.

[16] Collins, E.A, Bares, J., and Billmeyer, F.W. (1973) Experiments in Polymer Science, John Wiley & Sons

[17] Wolman, M. (1975). Polarized light microscopy as a tool of diagnostic pathology. Journal of Histochemistry & Cytochemistry, 23(1), 21-50.

On the other hand, the object can become difficult to see when aligned parallel to one of the two polarization planes.[18]

The aforementioned thickness variation resultant from microtoming, as well as the tendency of an anisotropic fiber to tear away from a surrounding matrix, can create edge artifacts under polarized light. An example of such an artifact is displayed in Figure 11 (as well as previous Figures 6 and 7), which is a micrograph of a non-oxidized (no possible "bark") exemplar PROLENE mesh fiber subjected to the H&E staining protocol. In Figure 11A, the fiber is shown under polarized light, and a dark ring of false "bark" is visible on a portion of the fiber exterior. Figures 11B and C are the same region at higher magnification, with and without the polarizer, respectively.

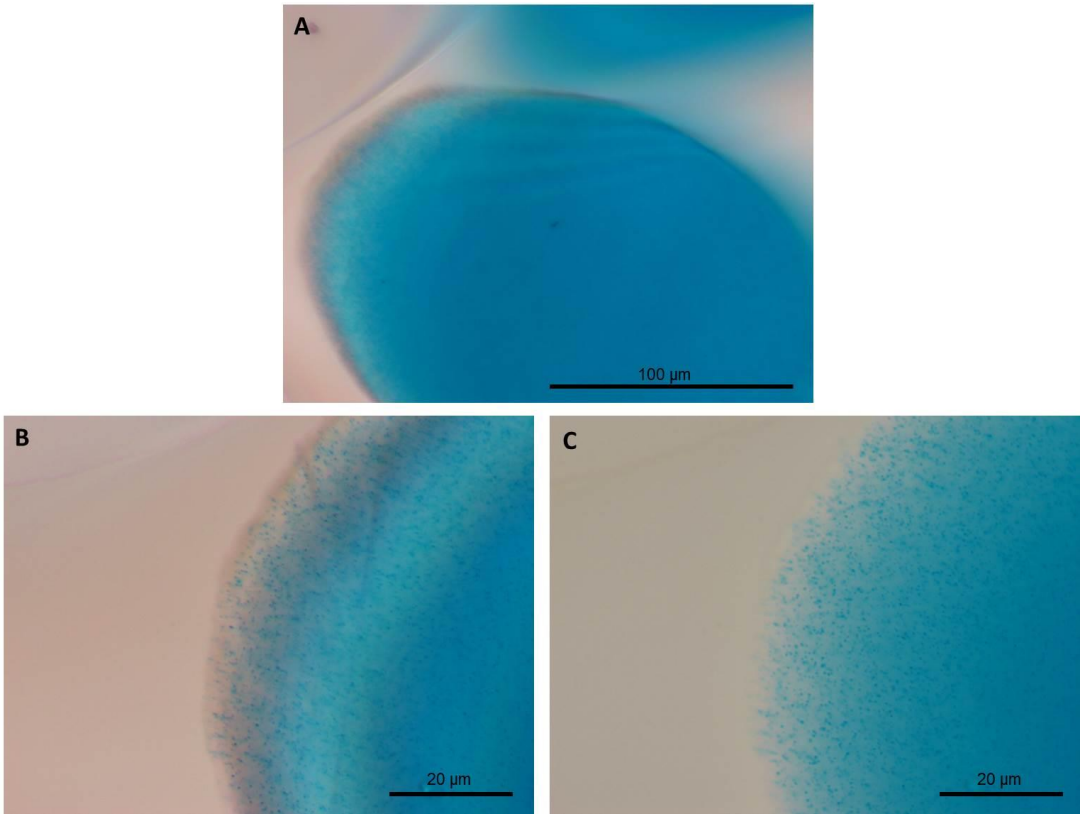

Figure 11. Exemplar, unoxidized, PROLENE mesh after staining with H&E. Images A and B were acquired with polarized light, image C was obtained without polarized light.

[18] Ibid.

Mechanical Behavior of PROLENE Fibers

Dr. Iakovlev opines that oxidation of the PROLENE mesh causes the formation of "a continuous brittle sheath around the mesh filaments contributing to mesh stiffening while the contracting forces acted to deform the mesh. Additionally, degradation of a substance indicates its breakdown into smaller molecules, and in cases of implanted materials, the products of degradation are released into the tissue adding to the complex pathological interactions between the mesh and the human body."[19] Dr. Iakovlev also notes that his alleged "bark" layer showed large nanocavities (cracks) that indicate brittleness.

From a fundamental polymer science perspective, Dr. Iakovlev's above-stated opinions are flawed for a number of reasons. First, if we assume, for sake of argument, that the "bark" layer is more stiff then the underlying material, if it is filled with cracks (or nanopores and nanocavities as Dr. Iakovlev calls them), it is by definition discontinuous and therefore mechanistically cannot contribute to an increase in stiffening.[20] Dr. Iakovlev cannot have it both ways, either the material is stiff and uniform and leads to mesh stiffening, or it cracks and forms pores and traps dyes; the two are mutually exclusive. Second, if the PROLENE is actually being broken down into smaller molecules, it will tend to become less stiff, not more. Again, Dr. Iakovlev cannot have it both ways (indeed, by stating degradation into smaller molecules leads to stiffening, Dr. Iakovlev underscores the flawed nature of his reasoning).

[19] Expert report of Dr. Vladimir Iakovlev dated May 23, 2015, p. 54

[20] Had he chosen to do so, with even a fundamental knowledge of mechanics, Dr. Iakovlev could have easily calculated that a continuous (without his observed pores and cracks) bark of the thickness he has measured could not meaningfully contribute to an increase in mesh stiffness.

Conclusion and Opinions

Based on my analysis, as well as my education, training and experience in mechanics of materials, polymer science and mechanical engineering, I have formed the following opinions to a reasonable degree of engineering and scientific certainty. If additional information becomes available, I reserve the right to supplement or amend any or all of these opinions.

- Dr. Iakovlev has not used any reliable scientific methods to conclusively determine that an outer oxidized PROLENE layer stains when exposed to H&E. Dr. Iakovlev's assertion that the mesh material has degraded *in vivo* is solely based on visual and microscopic observations of "bark" microcracking. He has conducted no quantitative experiments to confirm his visually based allegation that the mesh material is degraded or oxidized.

- Dr. Iakovlev has not performed any control experiments nor cited any scientific studies that support his belief that degraded PROLENE is capable of being histologically stained with H&E stains, and for these reasons, his conclusions are flawed and suspect.

- Through a series of controlled oxidation, microtoming and microscopy experiments, Exponent demonstrated that oxidized PROLENE meshes do not become stained with Hematoxylin & Eosin dyes. This fact is supported by polymer science first principles, given that PROLENE does not possess chemical groups amenable to binding with the H&E stain molecules.

- Artifacts can easily be introduced during sample preparation, sectioning, staining, and imaging, giving the appearance of darkened outer layers.

- A brittle outer layer will not contribute to the stiffness of the mesh if it is thin, cracked, and discontinuous. Dr. Iakovlev's opinion that a thin, cracked, porous outer layer causes an increase in mesh stiffness is not consistent with polymer science first principles and contradicted by the measured modulus data from Ethicon's seven year dog study.

Appendix A – Histology Protocols

Paraffin-embedded samples

1. Samples were processed and embedded in an automated tissue processor according to the following schedule:
2.

Processing Step	Incubating Solution	Number of Changes	Duration of Each Incubation Step
1	70% Reagent Alcohol	2	1 hour each
2	80% Reagent Alcohol	1	1 hour
3	95% Reagent Alcohol	1	1 hour
4	100% Reagent Alcohol	3	1.5 hours each
5	Xylene substitute (ProPar, Manufacturer)	3	1.5 hours each
6	Leica EM400 Paraffin wax	2	2 hours each

3. Tissues were embedded in paraffin blocks using Leica EM400 wax
4. The paraffin blocks were trimmed as necessary and cut at 4-6 μm-thick sections
5. The paraffin sections were briefly floated in a water bath set to 40-45^{o}C to remove wrinkles and allow them to flatten
6. The sections were mounted onto adhesive-coated glass slides, then air-dried for 30 minutes and baked in a 45-50^{o}C oven overnight

7. Paraffin-embedded samples were stained using an automated stainer programmed with the following protocol:

Processing Step	Incubating Solution	Duration of Each Incubation Step
1	65°C	10 min
2	Xylene	3 min
3	Xylene	2 min
4	Xylene	2 min
5	100% Alcohol	1 min
6	100% Alcohol	1 min
7	95% Alcohol	1 min
8	Water	1 min
9	Harris Hematoxylin	10 min
10	Wash station	1 min
11	Acid Alcohol	30 sec
12	Water	2 min
13	Ammonia Water	1 min
14	Water	1 min
15	Eosin	2 min
16	100% Alcohol	1 min
17	100% Alcohol	1 min
18	100% Alcohol	1 min
19	Xylene	1 min
20	Xylene	1 min

Resin-embedded samples

1. Samples were processed and embedded in an automated tissue processor according to the following schedule:

Processing Step	Incubating Solution	Number of Changes	Duration of Each Incubation Step
1	70% Reagent Alcohol	2	1 hour each
2	80% Reagent Alcohol	1	1 hour
3	95% Reagent Alcohol	1	1 hour
4	100% Reagent Alcohol	3	1.5 hours each
5	Technovit 7200	3	3 hours each

2. The resin samples were polymerize using a visible light polymerization unit
3. The blocks were trimmed as necessary, cut using a diamond saw blade, then ground and polished to approximately 50 μm thickness
4. Paraffin-embedded samples were stained using an automated stainer programmed with the following protocol:

Processing Step	Incubating Solution	Duration of Each Incubation Step
1	Water	1 min
2	Harris Hematoxylin	10 min
3	Water	1 min
4	Acid Alcohol	30 sec
5	Water	1 min
6	Ammonia water	1 min
7	Water	1 min
8	Eosin	1 min
9	Water	30 sec